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Inhibitory Action of Tetrathionate Enrichment Broth

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Tetrathionate enrichment broth is a complex mixture of salts including iodides and other polythionates, but only thiosulfate (0.0736 M) and tetrathionate (0.0236 M) in combination were toxic for *Escherichia coli*. Individually, these two salts were not lethal. The lethal action of this thiosulfate-tetrathionate mixture affected only growing cells. A possible relationship between the lethality of the thiosulfate-tetrathionate mixture for a culture and its ability to reduce tetrathionate is suggested.

When isolating salmonellae from sparsely infected material, it is necessary to inoculate the material into enrichment media which allow the growth of salmonellae while restricting the undesirable coliform and other gram-negative bacteria. The enrichment media most often used are the selenite broth of Leifson (7) and the tetrathionate broth of Muller (11). Although these broths have been widely used and often modified, their mode of action is still unknown. Further improvements involving these media could be more effectively developed if the mechanism of inhibition was more fully understood.

Pollock and co-workers (4–6, 16, 17), in a series of studies on the inhibition by tetrathionate broth, concluded that tetrathionate was the most important constituent of the medium. They found that most salmonellae and *Proteus* reduced tetrathionate to thiosulfate, whereas *Escherichia coli* and the shigellae did not. Tetrathionate reductase was demonstrated in washed cells and shown to be adaptive. It was suggested that tetrathionate acted as an alternative hydrogen acceptor to oxygen and therefore extended the log phase of growth. LeMinor (8) and Papavassiliou et al. (13) examined numerous *Enterobacteriaceae* cultures for tetrathionate reductase and found that most salmonellae possessed it whereas *E. coli* did not. They suggested that possession of this enzyme could prove useful in differentiating members of the enteric group.

Several exceptions to the relationship between the ability to reduce tetrathionate and the ability to grow in tetrathionate broth have, however, been observed (4, 22). Although numerous combinations of thiosulfate and tetrathionate were examined for inhibition of growth after 18 to 24 hr, no early growth measurements were made.

This paper is a report on the relationship of tetrathionate reductase production by both growing and resting cells of several cultures of *Enterobacteriaceae* to their inhibition by tetrathionate broth. Further, the basic components of the medium and the polythionates formed by their interaction have been examined to determine their role in this inhibition.

MATERIALS AND METHODS

Cultures. The salmonellae were obtained from Alice Moran, Consumer and Marketing Service, USDA (AM), and from H. Ng, Western Utilization Research and Development Division (WU). Other cultures were obtained from Z. J. Ordal, University of Illinois (UI), and from our own culture collection (EU). Appropriate dilutions of 24-hr cultures grown in Trypticase Soy Broth (BBL) were used as inocula for all studies.

Growth media. The basal medium for all studies was 0.5% proteose-peptone (Difco) plus buffer. When the medium contained thiosulfate and tetrathionate in the proportions found in tetrathionate broth, a white colloidal-sulfur precipitate, indistinguishable from growth, developed. In these cases, 1% $CaCO_3$ was used as buffer. If growth could be measured turbidometrically, phosphate buffer (*p*H 7.6) was used at a final concentration of 0.05 M. The basal medium including any salts except the thionates was sterilized by autoclaving.

Growth was measured either by turbidity in a Beckman model B spectrometer at 540 nm or by surface plate counts on Tryptic Soy Agar (TSA; Difco). Dilutions were made in 0.1% peptone (Difco) water. The plates were incubated at 37 C for 24 hr and counted. Negative plates were incubated longer to check for slow growing colonies.

In studies in which salmonellae and *E. coli* were

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mixed, the viable *Salmonella* count was followed on Brilliant Green Agar (Difco) and the viable *E. coli*, by the most probable number method in Lactose Purple Broth (Difco).

Preparation of thionates. Sodium tetrathionate ($Na_2S_4O_6 \cdot 2H_2O$) was prepared from sodium thiosulfate by iodine oxidation as suggested by Mellor (10). Potassium tetrathionate (K & K Laboratories, Plainview, N.Y.) was used in some studies.

Potassium trithionate ($K_2S_3O_6 \cdot 3H_2O$) and potassium pentathionate ($K_2S_5O_6 \cdot 3H_2O$) were prepared by the method of Palmer (12). All thionates were stored at −10 C in a desiccator. When used in growth or inhibition studies, they were prepared double-strength, sterilized by membrane filtration, and added to double-strength basal broth.

The purity of the thionates was checked by the method of Jay (3) and by paper chromatography (20). The paper chromatography method also was employed to quantitate the thionates formed by the thiosulfate-catalyzed breakdown of tetrathionate. The intensity of the spots was measured by procedures of Pollard et al. (15) by using a TLC Densitometer System (Photovolt Corp., New York). Because of the instability of the thionates, their purity was checked frequently by paper chromatography.

Tetrathionate reduction. Tetrathionate reductase was measured by two methods. (i) The method of Pollock and Knox (16) is an iodometric titration of the thiosulfate released from tetrathionate by washed cells grown on Tryptic Soy Agar (Difco). In this method, suspensions were mixed with tetrathionate and mannitol, and samples were titrated periodically. Induction time was calculated in the same manner as lag time (2). (ii) In the cultural method of LeMinor (8), a basal medium containing sodium tetrathionate (0.722%) is inoculated with the test organisms. At 24 hr, the broth is titrated with standard iodine solution. The results are compared to the control and expressed as either plus or minus. Some of the cultures also were tested for trithionate and pentathionate reduction by this method.

Resistance to tetrathionate. The gradient plate method (23) was employed to measure resistance to tetrathionate. The bottom layer was phosphate basal broth plus 1.5% agar (Difco) plus 2× tetrathionate (1.422%). The upper layer of phosphate basal broth plus agar was tempered, inoculated with the test organism, and poured over the slanted bottom layer. The length of growth along the concentration axis is a measure of the inhibitory concentration.

Temperature. The tetrathionate reduction and gradient plate studies were performed at both 37 and 43 C. All other studies and incubations were at 37 C.

Salts study. Tetrathionate broth base contains sodium thiosulfate which is converted to tetrathionate upon the addition of iodine dissolved in potassium iodide according to the reaction: $2Na_2S_2O_3 \cdot 5H_2O + I_2 + [KI] \rightarrow Na_2S_4O_6 \cdot 2H_2O + 2NaI + [KI]$. In practice, 0.1208 moles of sodium thiosulfate is reacted with 0.0236 moles of iodine dissolved in 0.0301 moles of potassium iodide. This yields 0.0236 moles of sodium tetrathionate plus 0.0472 moles of sodium iodide and 0.0301 moles of potassium iodide, with 0.0736 moles of sodium thiosulfate remaining unreacted. The molarities of sodium thiosulfate were calculated on the assumption that the salt used in preparing the medium is the pentahydrate. The four salts, sodium tetrathionate, sodium thiosulfate, potassium iodide, and sodium iodide, were tested for their effect on various bacteria.

Paper chromatography of the mixture of thiosulfate and tetrathionate showed that the thiosulfate catalyzed the breakdown of tetrathionate to other thionates. These thionates were quantitated, synthesized (12), and added to the basal broth to check their lethality.

Since sulfite is the first product formed when thiosulfate and tetrathionate react (20), the tetrathionate-thiosulfate mixture also was examined for the presence of sulfide and sulfite. Although no free sulfite was found, its lethal effect also was examined in phosphate basal broth.

RESULTS

Tetrathionate reduction. Figure 1 is representative of the responses obtained with different cultures when washed cells were added to the tetrathionate-mannitol suspension. The induction times obtained by this method for 63 strains of bacteria, primarily *Enterobacteriaceae*, are given in Tables 1 and 2. Most of the cultures were able to reduce tetrathionate, although 12 or more hours was required for some. Salmonellae,

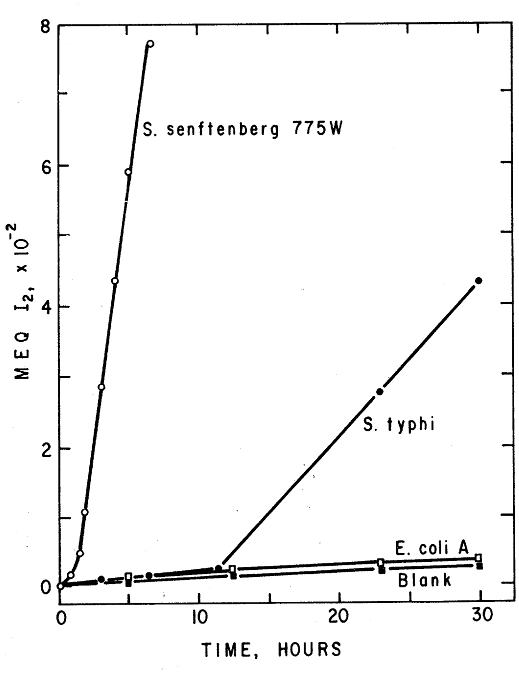

FIG. 1. *Tetrathionate reduction by washed suspensions of bacteria.*

TABLE 1. *Induction times for tetrathionate reduction by various bacteria found to be positive by the cultural method*

Organism	Induction time (hr)	Organism	Induction time (hr)
Salmonella senftenberg 775W	1	*S. chester* AM17	2
S. derby AM20	1	*S. anatum* AM78	2
S. derby AM21	1	*S. cubana* AM207	2
Pseudomonas aeruginosa EU314	1	*S. heidelberg* AM16	2
Escherichia coli D EU306	1	*S. typhimurium* AM10	3
Arizona sp. EU313	1	*S. typhimurium* AM11	3
S. tennessee EU317	1	*S. meleagridis* AM209	3
Salmonella sp. EU315	1.5	*S. saintpaul* AM108	3
Salmonella sp. EU316	1.5	*S. paratyphi* B AM3	3
S. typhimurium EU318	1.5	*S. oranienburg* AM42	4
S. bredeney AM112	1.5	*S. montevideo* AM46	4
S. newport EU320	1.5	*Klebsiella* sp. EU309	4
S. typhimurium EU319	1.5	*Proteus* sp. I EU310	6.5
S. thompson var. *berlin* AM40	1.5	*Proteus* sp. II EU311	7
S. thompson AM39	2	*Proteus* sp. III EU312	7
S. senftenberg WU-S8	2	*S. typhi* AM60	11.5
S. blockley WU2004	2	*S. typhimurium* AM12	12
S. typhimurium AM9	2	*S. infantis* AM165	12

TABLE 2. *Induction times for tetrathionate reduction by various bacteria found to be negative by the cultural method*

Organism	Induction time (hr)	Organism	Induction time (hr)
Pseudomonas sp. EU92	0.5	*Sarcina lutea* EU112	11.5
P. fragi EU43	0.5	*S. abortusovis* AM27	11.5
P. ovalis EU36	0.5	Gram-negative rod no. 2	11.5
Serratia marcescens EU279	5	*S. pullorum* AM75	12
Salmonella enteritidis	5.25	*S. paratyphi* A AM1	12
Aerobacter aerogenes EU109	5.5	*Shigella* sp. EU321	12
A. aerogenes EU110	5.5	*Bacillus subtilis* UI-AI	12
Escherichia coli EU106	6	*B. cereus* UI-T	12
Gram-negative rod no. 1	6.5	*A. aerogenes* EU300	23
S. choleraesuis AM34	7	*A. aerogenes* EU301	23
S. choleraesuis var. *kunzendorf* AM36	7	*Enterobacter* sp. EU302	23
E. coli B EU304	7	*E. coli* CC EU308	23
E. coli E EU307	7	*E. coli* C EU305	>30
		E. coli A EU303	>30

with few exceptions, had very short induction times. The induction time was independent of the weight of cells, although the rate was proportional to cell mass once reduction had begun. An excess of thiosulfate equal to that present in the complete enrichment broth did not affect either induction time or rate of reduction by selected cultures. Incubation at 43 C did not appreciably affect induction time.

Also given in Tables 1 and 2 are the results from the cultural method of determining tetrathionate reductase. In general, bacteria with short induction times for washed cells were also positive by this method (Table 1). Several cultures were examined by the cultural method for their ability to reduce tri- and pentathionate; cultures positive for tetrathionate reduction were positive for the other two thionates and vice versa.

The *Pseudomonas* sp. and *Aerobacter* sp. were exceptions in that they were negative by the cultural method, but had short induction times (Table 2). When checked at frequent intervals during the 24-hr incubation period, no iodine-reacting compounds were detected even though the cells grew well.

Gradient plate studies. All cultures, tested at both 37 and 43 C, grew at least half way across the gradient plates. This indicated the ability to grow in a concentration of tetrathionate equal to that found in tetrathionate broth.

Effect of enrichment-broth constituents on growth. The gradient plate studies and the ability of most cultures to reduce tetrathionate suggested that the inhibitor in tetrathionate broth was not the tetrathionate alone. Therefore, the ability of various combinations of the four salts in tetrathionate broth to inhibit *S. derby* and *E. coli* EU106 was examined. The combinations were: basal (no salt), NaI (0.0472 M), KI (0.0301 M), thio ($Na_2S_2O_3 \cdot 5H_2O$, 0.0736 M), tet ($Na_2S_4O_6 \cdot 2H_2O$, 0.0236 M), NaI-KI, KI-thio, thio-tet, NaI-tet, NaI-thio, KI-thio, NaI-KI-thio, NaI-KI-tet, NaI-thio-tet, KI-thio-tet, and NaI-KI-thio-tet. The only combinations showing inhibition of *E. coli* EU106 were those containing both thiosulfate and tetrathionate. *S. derby* was not inhibited by any combination. The viable cell count for *S. derby* increased from 10^4 to 2×10^8 per ml in 20 hr. The viable cell count for *E. coli* EU106 increased in a similar manner, except in those combinations containing both thiosulfate and tetrathionate. In this instance the viable cell count at 20 hr was more than four log cycles below that of the basal. Since the combination of thiosulfate and tetrathionate markedly inhibited *E. coli* EU106, while not affecting *S. derby*, these two salts were examined for their effect on the growth of several other cultures. Representative data are presented in Fig. 2. Data similar to those for *E. coli* A were obtained for seven other strains of *E. coli*, *Shigella* sp., *S. paratyphi* A, and *S. choleraesuis*. Data similar to those obtained for *S. typhimurium* were obtained for six other strains of *Salmonella*, *S. paratyphi* B, *Pseudomonas aeruginosa*, two strains of *Proteus*, four strains of *Aerobacter*, *Klebsiella* sp., and *Arizona* sp. In most instances, the viable cell count at 12 hr was the same as that at 20 hr. Results were similar with the commercially prepared tetrathionate and the one prepared in our laboratory. It also was observed that tetrathionate, although not inhibiting *E. coli*, stimulated the total growth of salmonellae.

Figure 3 shows the typical response of a mixture of salmonellae and *E. coli* in basal broth with thiosulfate and tetrathionate. Viable *E. coli* declined markedly, whereas the salmonellae showed typical growth.

Effect of components of thiosulfate-tetrathionate mixture on growth of E. coli. When thiosulfate is mixed with tetrathionate in the proportions found in the complete broth, 0.0736 moles and 0.0236 moles, respectively, colloidal sulfur and polythionates are formed (1, 15, 20). Paper chromatography of the solution after mixing the tetrathionate and thiosulfate showed that it did contain, in addition to the starting compounds, tri- and pentathionates and traces of compounds

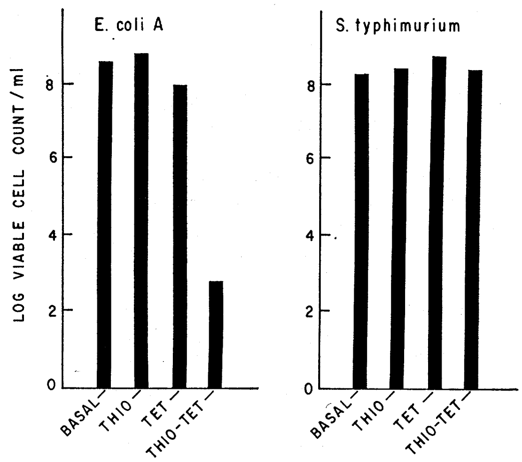

FIG. 2. *Influence of thiosulfate and tetrathionate on the growth of E. coli A and S. typhimurium. Viable cell count at 20 hr. Thiosulfate concentration, 0.0736 M; tetrathionate concentration, 0.0236 M.*

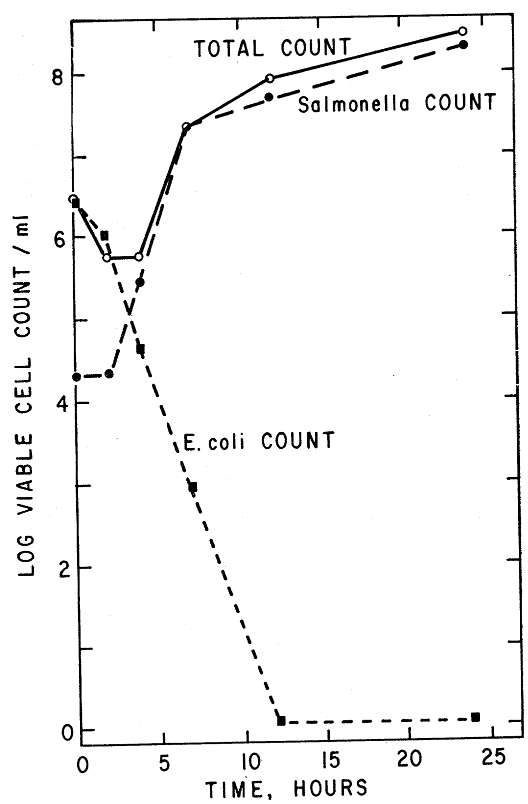

FIG. 3. *Viable cell count of salmonellae (S. derby and S. typhimurium) and E. coli (A and B) in basal broth containing 0.0736 M thiosulfate and 0.0236 M tetrathionate.*

assumed to be hexa-, hepta-, and octathionates on the basis of their position on the chromatogram. A quantitative analysis of the mixture is shown in Table 3. Paper chromatograms showed that the various thionates were formed within 2 hr after mixing.

Trithionate, pentathionate, and a mixture of tri-, tetra-, and pentathionates were found to have no effect on the growth of *E. coli*. The higher thionates were not tested due to difficulties in isolation and purification (18).

The mixture was examined for other sulfur compounds by the column technique of Levinthal and Schiff (9), and no detectable amounts of sulfide or sulfite were found. However, since sulfite is the first compound formed when thiosulfate and tetrathionate react (20), its effect on *E. coli* and salmonellae was investigated. When added to the basal medium in concentrations as high as 0.04 M, the only effects were that the lag times of both cultures increased slightly and their maximum populations were decreased slightly. Removal of colloidal sulfur from the thiosulfate-tetrathionate mixture by filtration did not affect the subsequent lethality of the filtrate when added to basal medium and inoculated with *E. coli.*

The data in Table 3 indicate that thiosulfate catalyzed the breakdown of tetrathionate. To assess the quantitative relationships, 1.83 grams of thiosulfate per liter (0.00736 M) was mixed with 7.22 grams of tetrathionate per liter (0.0236 M) in basal broth and allowed to react for 40 hr and analyzed by paper chromatography. Quantitative analysis showed that this low thiosulfate mixture contained the same concentrations of thionates as the regular thiosulfate-tetrathionate mixture (Table 3). However, this low thiosulfate mixture showed no lethality towards *E. coli*. A mixture of thiosulfate and low tetrathionate (1/10×) also had no lethal effect on *E. coli*.

The effect of adding graded amounts of thiosulfate to tetrathionate in basal broth on the viable count is given in Fig. 4. These data indicate that the thiosulfate concentration must be at least 0.06 M to have any great effect. The maximum lethal effect on *E. coli* occurs at a 3 to 1 molar ratio of thiosulfate-tetrathionate (0.0736 M and 0.0236 M, respectively).

TABLE 3. *Quantitative analysis of the thiosulfate-tetrathionate mixture*

Compound	Before mixing (moles/liter)	After mixing (moles/liter)
Thiosulfate	0.0736	0.0662
Trithionate		0.0121
Tetrathionate	0.0236	0.0131
Pentathionate		0.00103
Hexathionate		Trace
Heptathionate		Trace
Octathionate		Trace

Studies with growing and nongrowing E. coli. Since the thiosulfate-tetrathionate mixture was lethal to growing *E. coli*, its effect on nongrowing cells was investigated. *E. coli* A was grown for

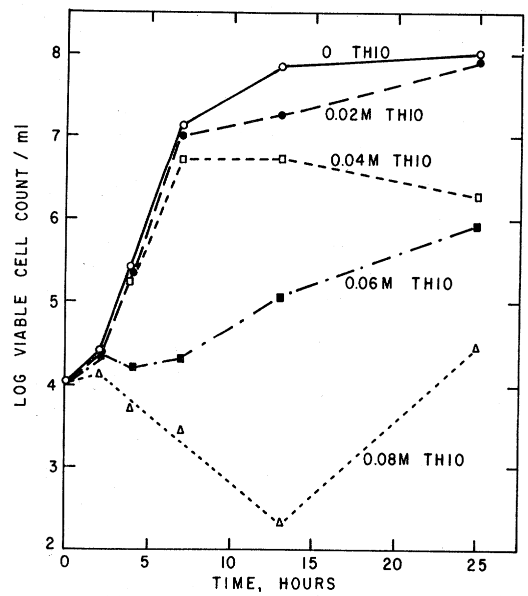

FIG. 4. *Influence of different concentrations of thiosulfate (M thio) in basal broth containing 0.0236 M tetrathionate on the viable cell count of E. coli A.*

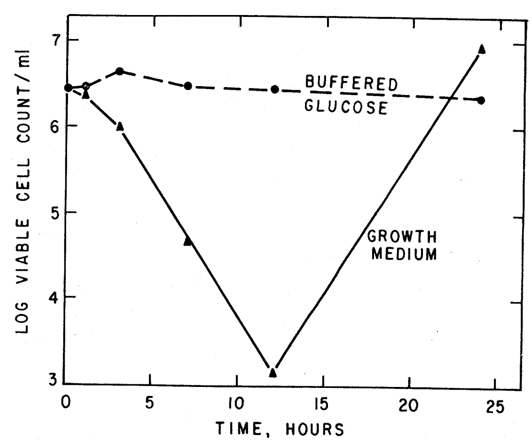

FIG. 5. *Influence of the thiosulfate-tetrathionate mixture on washed cells of growing and nongrowing E. coli A.*

24 hr in phosphate basal broth and washed once with 0.05 M phosphate buffer (*p*H 7.6). The cells were resuspended in buffer and added to 0.5% glucose (nongrowing cells) or to 0.5% proteose-peptone (growing cells). Thiosulfate and tetrathionate were added, and the suspensions were incubated at 37 C. Periodically, samples were placed on XL agar (Difco). After 24 hr, cells in buffered glucose showed no change, whereas the growing cells showed the typical decline and regrowth (Fig. 5). Cells in buffer alone also showed no change in number.

DISCUSSION

When washed cells were incubated with tetrathionate, there was poor correlation between induction time and inhibition in tetrathionate broth except with certain salmonellae and *E. coli*. With the cultural method for detecting reduction, a closer relationship was observed, but there were several exceptions. When these observations are coupled with the lack of a lethal action of tetrathionate alone on any of the cultures, it appears doubtful that the production of tetrathionate-reducing enzyme is the primary reason for the inhibitory action of tetrathionate broth.

The stimulatory effect of tetrathionate on the growth of bacteria which reduce it as reported by Pollock and co-workers (4, 5) was also observed in our studies. As they noted, however, this was not an effect on growth rate, but on total growth, and was never more than one log cycle.

When the various combinations of compounds that are either added to or produced in tetrathionate broth were investigated, the lethal effect was observed only when both tetrathionate and thiosulfate were present. Although it was demonstrated that other polythionates were formed when tetrathionate and thiosulfate were mixed, the tri-, tetra-, and pentathionates were shown to have no effect. The higher thionates were not available as pure compounds for testing, but their possible involvement was eliminated when a low level of thiosulfate was mixed with tetrathionate. The quantities of polythionates produced by this combination were essentially the same as found in the regular tetrathionate broth, yet no lethality occurred. The report by Smith (21) that di-, tri-, and pentathionates would relieve selenite-inhibited growth of *E. coli* is added evidence that these compounds do not have a significant role in the inhibitory action of tetrathionate broth.

Knox et al. (5) found that iodide-free tetrathionate was less inhibitory than tetrathionate made from iodine and thiosulfate, whereas Muller (11) got better inhibition of *E. coli* when he used iodide-free tetrathionate. Our data indicated that iodides have no significant role in the lethal action of tetrathionate broth.

Preuss (19) proposed a new tetrathionate broth containing only calcium tetrathionate. However, when mixed cultures containing salmonellae or *Proteus* are inoculated into it, reduction of the tetrathionate will occur and thus it would become like regular tetrathionate broth, i.e., a lethality due to thiosulfate and tetrathionate. This medium might prove better in that reduction would begin later in the enrichment period either because of few cells or the slow growth of injured cells. This late reduction would then yield the thiosulfate necessary to kill susceptible cells.

Proteus, *Enterobacter*, and *P. aeruginosa* are similar to salmonellae in not being inhibited by the thiosulfate-tetrathionate mixture. These organisms also come through the tetrathionate enrichment and present problems during the isolation of salmonellae from meat and meat products (Alice Moran, *personal communication*).

The data in Fig. 4 and 5 show the typical decline and regrowth of *E. coli* in thiosulfate-tetrathionate broth. The possibility that this regrowth is due to selection of a mutant was tested by picking colonies from the TSA plate at 24 hr and reinoculating them into thiosulfate-tetrathionate broth. These cultures showed the typical decline and regrowth and thus we discounted the idea of a mutant. This regrowth could be caused by a decrease in the toxic substance(s). Although *E. coli* does not reduce tetrathionate, there is a chemically caused decrease in the tetrathionate concentration. This chemical change alters the importance of the tetrathionate-thiosulfate ratio. Thus we have further support for the importance of the tetrathionate-thiosulfate ratio.

The lethality of tetrathionate broth is directly related to the concentrations of thiosulfate and tetrathionate in the medium. Decreasing the concentration of either salt reduces the lethal action of the mixture. Concentrations of 0.0736 M and 0.0236 M of thiosulfate and tetrathionate, respectively (3:1 ratio), appear to be optimal. The ability of a microorganism to reduce tetrathionate may play a role in decreasing the inhibition for certain species. When part of the tetrathionate is reduced, the ratio would be altered.

The lethality is a growth-related phenomenon since no killing occurred with nongrowing cells in the presence of the thiosulfate-tetrathionate mixture (Fig. 5). The actual mechanism of this lethal action is not known. However, tetrathionate is known to react with free sulfhydryl groups of enzymes and to cause their inactivation (14). Thiosulfate can also react with sulfhydryl groups (18). Thus, it is suggested that tetrathionate broth interferes with the synthesis, the activity, or both, of sulfur-containing enzymes or cell wall and membrane components.

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LITERATURE CITED

1. Blasius, E., W. Burmeister, and G. Wermbter. 1961. Untersuchung der Wackenroderschen Reaktion mit Hilfe der radiopapierschromatographischen Method. III. Z. Anorg. Allgem. Chem. **313**:254–261.
2. Finn, R. K. 1955. Measurement of lag. J. Bacteriol. **70**:352–353.
3. Jay, R. J. 1953. Determination of polythionic acids. Anal. Chem. **25**:288–290.
4. Knox, R. 1945. The effect of tetrathionate on bacterial growth. Brit. J. Exp. Pathol. **26**:146–150.
5. Knox, R., P. G. H. Gell, and M. R. Pollock. 1943. The selective action of tetrathionate in bacteriological media. J. Hyg. **43**:147–158.
6. Knox, R., and M. R. Pollock. 1944. Bacterial tetrathionase: adaptation without demonstrable cell growth. Biochem. J. **38**:299–304.
7. Leifson, E. 1936. New selenite enrichment media for the isolation of typhoid and paratyphoid (*Salmonella*) bacilli. Amer. J. Hyg. **24**:423–432.
8. LeMinor, L. 1967. Distribution de la tetrathionate-reductase chez divers serotypes de *Salmonella*. Ann. Inst. Pasteur **113**: 117–123.
9. Levinthal, M., and J. A. Schiff. 1968. Studies of sulfate utilization by algae. 5. Identification of thiosulfate as a major acid-volatile product formed by a cell-free sulfate-reducing system from *Chlorella*. Plant Physiol. **43**:555–562.
10. Mellor, J. W. 1930. Comprehensive treatise on inorganic and theoretical chemistry, vol. 10. Longmans Green, London.
11. Muller, J. 1923. Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. Compt. Rend. Soc. Biol. **89**:434–437.
12. Palmer, W. G. 1954. Experimental inorganic chemistry. Cambridge University Press, London.
13. Papavassiliou, J., V. Samaraki-Lyberopoulou, and G. Piperakis. 1969. Production of tetrathionate reductase by *Salmonella*. Can. J. Microbiol. **15**:238–240.
14. Parker, D. J., and W. S. Allison. 1969. The mechanism of inactivation of glyceraldehyde 3-phosphate dehydrogenase by tetrathionate, *o*-iodosobenzoate, and iodine monochloride. J. Biol. Chem. **244**:180–189.
15. Pollard, F. H., G. Nickless, D. J. Jones, and R. B. Glover. 1964. Chromatographic studies on sulphur compounds. III. Quantitative analysis of a mixture containing thiosulphate and polythionates by paper chromatography. J. Chromatogr. **15**:407–419.
16. Pollock, M. R., and R. Knox. 1943. Bacterial reduction of tetrathionate. Biochem. J. **37**:476–481.
17. Pollock, M. R., R. Knox, and P. G. H. Gell. 1942. Bacterial reduction of tetrathionate. Nature (London) **150**:94.
18. Postgate, J. R. 1963. The examination of sulphur auxotraphs: a warning. J. Gen. Microbiol. **30**:481–484.
19. Preuss, H. 1949. Uber eine neue Tetrathionat-Anreicherung. Z. Hyg. **129**:187–214.
20. Skarzynski, B., and T. W. Szczepkowski. 1959. Oxidation of thiosulphate by *Thiobacillus thioparus*. Nature (London) **183**:1413–1414.
21. Smith, H. G. 1959. On the nature of the selective action of selenite broth. J. Gen. Microbiol. **21**:61–71.
22. Smith, H. W. 1952. The evaluation of culture media for the isolation of Salmonella from faeces. J. Hyg. **50**:21–36.
23. Szybalski, W., and V. Bryson. 1952. Genetic studies on microbial cross resistance to toxic agents. I. Cross resistance of *Escherichia coli* to fifteen antibiotics. J. Bacteriol. **64**: 489–499.